RESEARCH ARTICLE

Bumadizone calcium dihydrate microspheres compressed tablets for colon targeting: formulation, optimization and *in vivo* evaluation in rabbits

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Abstract

The objective of this study was the development of a colon-targeted microspheres which were compressed into tablets containing the non-steroidal anti-inflammatory bumadizone calcium dihydrate. A 3^2 full factorial design was adopted for the evaluation of the prepared microspheres. The effect of two independent variables namely polymer type (Eudragit RS100, ethyl cellulose and cellulose acetate butyrate), and drug: polymer ratio (1:1, 9:1 and 18:1) was studied on the entrapment efficiency and *in vitro* drug release for 12 h. Colon targeting aims to minimize the release of the drug off target area (pH 1.2 and 6.8) and to maximize the release of the drug in target area (pH 7.4). Candidate formulae were compressed into core tablets and colon targeting was achieved using the enzyme-dependent polymer (pectin) as coat in three different concentrations 50, 75 and 90%. Candidate formula F15 (microspheres prepared using BDZ:CAB in a ratio of 18:1 and compressed into tablets using 50% pectin and 50% Avicel in the coat) was able to adequately modulate drug release avoiding drug release in the gastric ambient, and reaching the colonic targeting where 99.7% release was achieved within 12 h following zero-order model. *In vivo* studies showed that F15 achieved significant decrease in myeloperoxidase activity and inflammation with delayed T_{max} (4 h) and lower C_{max} (2700 ng/ml) when compared to marketed product.

Introduction

Inflammatory Bowel Diseases (IBD) are an immune-mediated chronic or relapsing disorders of the gastrointestinal (GI) tract. They are characterized by a chronic intestinal inflammatory process (Klein & Eliakim, 2010). They are gaining more and more attention. This is due to the fact that there is increased incidence of IBD in the past 50 years, up to 120–200/100 000 persons and 50–200/100 000 persons for ulcerative colitis (UC) and Crohn's disease (CD) respectively (Cosnes et al., 2011). Also for the increased evidence, patients with IBD are at increased risk of developing colorectal cancer (Munkholm, 2003).

Site-specific delivery of drugs to the site of action has the potential to reduce side effects and to increase pharmacological response. One of the seemingly interesting areas to target drugs through oral route is the colon. Colon targeting denotes dosage forms that are able to sense the arrival to the colon and release the drug upon activation to achieve an optimum pharmacological action together with decreasing drug side effects (Vandamme & Lenourry, 2002).

Different mechanisms for colon targeting have been adopted; namely the use of prodrugs (Oz & Ebersole, 2008) and the use of pH-dependent polymers. These polymers have ability to withstand an environment ranging from low pH (~1.2) to neutral pH (~7.5) for several hours (Singh, 2007). A third mechanism in colon targeting involves the presence of time-dependent polymers. In these systems, the site of drug release is decided by the transit time of a formulation in the GI tract (Singh, 2007). The abundance of microflora in the colon area has also been used to achieve colon targeting (Sinha & Kumria, 2003). Hence, enzymatically controlled delivery systems are considered a convenient approach for site-specific drug delivery to the colon where no drug release can occur unless the system arrives to the colon. This helped the use of moieties that are susceptible to these enzymes including pectin and guar gum (El Gamal et al., 2013). Designing the previous systems using multiple-units, like microparticles and nanoparticles, is preferred for many reasons (Abdallah et al., 2009). They are less likely to be affected by food and demonstrate more consistent absorption compared to single unit systems (Amrutiya et al., 2009). They are well retained and they have greater potential to provide a uniform spreading of the drug particles to the inflamed parts of GI tract, which is advantageous for the topical therapy of inflamed bowel diseases. In addition, the residence time of other delivery systems like tablets or pellets is usually

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decreased by diarrhea, a common symptom of IBD (Meissner et al., 2006)

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of chronic inflammatory conditions. In addition, they showed that a promising activity for the prevention and treatment of colitis but with tendency to cause gastric bleeding and form ulceration in the gastric lining (El-kamel et al., 2008). Bumadizone calcium dihydrate (BDZ) is an acetic acid derivative NSAID. Formulation of BDZ into colon-targeted dosage forms will avoid these undesirable effects.

Literature search revealed that no previous studies were carried out to formulate colon-targeted dosage forms containing BDZ. The aim of this study was the development and evaluation of colon-targeted microspheres and compressing them into tablets containing BDZ to prevent drug release in the stomach, had an additional lag phase to retard drug release in the small intestine, and to deliver drug specifically to the colon to avoid gastrointestinal side effects of the drug and to increase the patient compliance. The promising dosage form was examined for its efficacy using acetic acid-induced rabbit colitis model in order to elucidate its usefulness as a specific drug delivery system for the treatment of ulcerative colitis. Finally, the pharmacokinetics of the drug was estimated following oral administration of the tablet to rabbits compared to marketed tablet.

Materials and methods

Materials

Bumadizone calcium dihydrate (BDZ) and Octomol® tablets (110 mg) were kindly provided by October Pharma, Egypt. Cellulose acetate butyrate (CAB) was obtained from Aldrich Chemical Company, Germany. Ethyl cellulose N100 (EC), pectin and acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Sigma Chemical Co., St. Louis, (USA). Avicel pH 101 (microcrystalline cellulose) was obtained from Morgan Chemical Industrial Company, Egypt. Eudragit® RS 100 was kindly provided from Degussa, Rohm GmbH and Co. KG, Pharma Polymer, Germany. Mannitol was generously supplied from Roquette, France. Poly vinyl alcohol (PVA; (Mw 88 000) and triethyl citrate were obtained from Acros Organics, Belgium. Dichloro methane was purchased from El Nasr pharmaceutical Chemical Company, Egypt. Pectinex (3000 FDU/ml), was purchased from Fluka Chemicals, Switzerland.

Preparation of BDZ-loaded microspheres

A 3^2 full factorial design was adopted to study the effect of two independent variables: (i) polymer type and (ii) drug-to-polymer ratio. Three time-dependent polymers, namely, Eudragit® RS100, ethyl cellulose and CAB and three drug-to-polymer ratios, namely, 1:1, 9:1 and 18:1 were used. Table 1 summarizes the independent variables along with their levels. Nine formulae were prepared as given in Table 2. PVA concentration, internal phase to external phase ratio and stirrer speed were kept constant and were selected based on preliminary studies (data not shown).

Microspheres were prepared according to quasi-emulsion solvent diffusion method (Jain et al., 2011) using the different variables. Briefly the calculated amount of the drug, polymer and 400 mg triethyl citrate (plasticizer) were dissolved in 20 ml dichloromethane (internal phase). The resulting solution was poured onto a 200 ml of 0.5% PVA aqueous solution (external phase) saturated with 10% sodium chloride and 3% calcium chloride (to prevent the escape of the drug to the external phase) and stirred at 500 rpm. Stirring continued for 8 h until dichloromethane has evaporated completely. The formed microspheres were then separated by filtration, washed 4–5 times with 75 ml distilled water and left to dry at room temperature for 24 h.

Characterization of the microspheres

Scanning electron microscopy

For morphology and surface characteristics, candidate formula was taken and fixed on a scanning electron microscopy (SEM) holder with double sided adhesive tape and coated with gold–palladium under an argon atmosphere at room temperature and then the surface morphology of the microspheres was studied by scanning electron microscope (SEM) using a JEOL JXA 840A (Tokyo, Japan).

Table 1. The experimental plan of the 3^2 factorial design used for preparation of BDZ microspheres.

Variables	Polymer type	Drug:polymer ratio
Levels	Eudragit RS100	1:1
	Ethyl cellulose	9:1
	CAB	18:1

Table 2. Mean particle size (μm), production yield, % entrapment efficiency, % of total drug released (mg%) 12 h and desirability of the prepared BDZ-loaded microspheres (mean ± SD, $n = 3$).

Formulation	Polymer	Drug:polymer ratio	Production yield (%)	Theoretical drug content (%)	Entrapment efficiency (%)	Mean particle size (μm ± SD)	Total release	Desirability
F1	Eu	1:1	79.31 ± 2.02	50	44 ± 10.36	90.81 ± 10.47	36.36	0.29
F2		9:1	75.69 ± 3.36	90	92.72 ± 5.23	106.46 ± 46.61	64.23	0.73
F3		18:1	67.38 ± 4.34	94.73	99.77 ± 1.25	142.23 ± 38.5	79.81	0.79
F4	Ec	1:1	75.35 ± 5.58	50	70.4 ± 6.65	111.95 ± 24.09	27.1	0.32
F5		9:1	71.49 ± 3.78	90	90.47 ± 2.23	125.14 ± 32.01	75.8	0.63
F6		18:1	75.41 ± 1.88	94.73	97.77 ± 0.77	138.64 ± 17.4	96.68	**0.87**
F7	CAB	1:1	45.45 ± 5.23	50	61.95 ± 7.78	139 ± 43.9	21.63	0.10
F8		9:1	58.81 ± 3.9	90	86.14 ± 1.25	145.91 ± 42.8	52.04	0.4
F9		18:1	80.2 ± 1.04	94.73	96.83 ± 1.22	147.95 ± 22.2	99.1	**0.96**

Eu, Eudragit; Ec, ethyl cellulose; CAB, cellulose acetate butyrate.

Particle size analysis

Particle size analysis was performed using laser diffraction (LD) technique. Samples were measured in distilled water; diameters and particle size distribution of the prepared microspheres were determined using Mastersizer Laser Diffractometer (Malvern Instruments, Malvern, Worcestershire, UK).

Determination of production yield and entrapment efficiency

The microspheres formed were collected and weighed. The percentage yield was determined using the following formula:

$$\frac{\text{weight of microspheres}}{\text{weight of solids added to the inner phase}} \times 100$$

Fifteen micrograms of the prepared microspheres were crushed and stirred with 100 ml phosphate buffer (pH 7.4) for 24 h. The extract was filtered and the drug was assayed spectrophotometrically (UV–VIS spectrophotometer, Shimadzu UV 1601 PC, Kyoto, Japan) at λ_{max} of 234 nm after doing the necessary dilution (Verma et al., 2010). To determine the entrapment efficiency the following formula was used:

$$\frac{\text{actual weight of the drug in 15 mg microspheres}}{\text{theoretical weight of the drug in 15 mg microsphere}} \times 100$$

Differential scanning calorimetry

Samples of the drug, blank microspheres and the candidate formula (F9) were heated in an aluminum pan at 5 °C/min in an atmosphere of nitrogen to 200 °C. The differential scanning calorimetry (DSC) thermograms were recorded on a differential scanning calorimeter (DSC-60, Shimadzu, Kyoto, Japan).

X-ray diffraction

X-ray diffraction (XRD) patterns of pure BDZ, blank CAB microspheres and candidate BDZ loaded microspheres were determined at a voltage of 40 kV and a current intensity of 30 mA at a scanning speed of 1 °C/min using a Philips PW 1729 X-ray diffractometer (Legroupe Interconnexion, Saint-Juire, Clubac, Canada).

In vitro drug release

Release of the drug from the microspheres was performed using USP dissolution tester type I (VK700 Dissolution Testing Station, Vankel Industries, Inc., NJ, USA). The baskets were covered by a piece of white muslin cloth (Lakshmana et al., 2009) to prevent the escape of the microspheres to the release media. Accurately weighed microspheres equivalent to 110 mg of the drug were used in the dissolution study. The basket was rotated at 100 rpm. *In vitro* release studies were carried out at $37 \pm 1 \,^\circ$C in 900 ml of 0.1 N HCl containing 1% PEG 400 (to maintain sink conditions) for a period of 2 h followed by release in Sorensen's phosphate buffer pH 6.8 for 2 h then Sorensen's phosphate buffer pH 7.4 for 8 h to simulate the pHs pertaining to the stomach, proximal and middle small intestine (duodenum and jejunum), and distal small intestine (ileum), respectively (Saboji et al., 2012). Aliquots were withdrawn in a predetermined time intervals, filtered then assayed spectrophotometrically for the drug at $\lambda_{max} = 232.6$ nm in pH 1.2 and λ_{max} 234 nm (Ali et al., 2012) in pH 6.8 and 7.4. The withdrawn samples were replaced with equal volume of the release media. The cumulative drug percent released was plotted against time. The experiments were performed in triplicate for each sample.

Statistical analysis

The experimental results regarding production yield, entrapment efficiency and *in vitro* release after 12 h were analyzed using Design Expert® 7.0 software (Design-Expert trial version 7; State-Ease Inc., Minneapolis, MN).

Preparation of compression coated colon-targeted tablets

Based on the previously mentioned characterization, and the results of the main effects of the adopted factorial design two candidate formulae, namely, F6 (18:1 drug to EC) and F9 (18:1 drug to CAB) were chosen. Both formulae showed maximum yield and entrapment efficiency and the required drug release profile (minimum drug release in pH 1.2 and pH 6.8 and maximum drug release in pH 7.4). Microspheres of candidate formulae containing 110 mg drug was mixed with 15% Avicel, 2% sodium lauryl sulfate and mannitol as diluent to a total weight of 200 mg and were compressed with an applied force of 4000 kg using a single punch tablet machine equipped with concave punch 10 mm in diameter forming the core tablet (tablet single punch press machine, Royal Artist, Bombay, India). The core tablets were compression-coated with 300 mg different coat mixtures (Table 3). Different pectin: Avicel mixture was used as outer shell for compression coating where pectin is considered as an enzyme

Table 3. Formulation of core and coat of different compression coated tablets for colon targeting.

Formula code	Polymer used	Drug to polymer ratio	Amount of microparticles weighed (mg)	15% avicel (mg)	2% Sodium lauryl sulfate (mg)	Mannitol (mg)	Pectin %	Avicel %
			Core*				Coat*	
F10	Ethyl cellulose	18:1	119	30	4	47	90	10
F11							75	25
F12							50	50
F13	CAB	18:1	120	30	4	46	90	10
F14							75	25
F15							50	50

Core*: 200 mg core tablet; Coat*: 300 mg coat tablet.

dependent polymer to achieve colon targeting. Fifty percent of coating material (150 mg) was placed in the die cavity, and the core tablet was placed in the center followed by addition of the remainder of the coating material. The coating material was compressed around the core tablet using round flat punches (12 mm) on the same tabletting machine. The mixture of powders prepared as core materials were evaluated for their flow properties using angle of repose method (Singh et al., 2012) and the prepared tablets were tested for weight variation, hardness, thickness and friability.

In vitro drug release

Release of the drug from the compression-coated colon-targeted tablets was performed like that of the prepared microspheres. The experiments tests were performed in triplicates. The release of the formulae that showed the required drug release profile (minimum drug release before target area pH 1.2 and pH 6.8 and maximum drug release in target area pH 7.4) were repeated in the presence of pectinase enzyme to simulate the colonic *in vivo* conditions.

Kinetic modeling of the release profiles

The release profiles of the drug were fitted to zero-order, first-order, Higuchi model and Korsmeyer–Peppas model (Costa et al., 2001).

In vivo study

Study design

In vivo study was done to compare the anti-inflammatory activity on colonic mucosa and the pharmacokinetics of BDZ from the candidate formula F15 and the marketed product Octomotol® tablets using a non-blind, two treatments and randomized design. The protocol of the study was approved by the Research Ethics Committee in the Faculty of Pharmacy, Cairo University, Egypt. The study was conducted in accordance with EC Directive 86/609/EEC for animal experiments.

Twelve New Zealand white rabbits of either sex weighing 2–2.5 kg were randomly distributed between four groups of equal number. Group I: normal animals, group II: animals with acetic acid induced ulcerative colitis, group III: animals with induced ulcerative colitis and treated with marketed tablets and group IV: animals with induced ulcerative colitis and treated with F15.

Induction of colitis

Colitis was induced in rabbits following the method described by Hathaway et al. (2012). Briefly, rabbits were fasted 24 h with free access to water before experimentation (Millar et al., 1996), a catheter was advanced rectally into the colon until its tip was 8 cm above the anus. The acetic acid/ethanol (1 ml of 5% acetic acid–30% ethanol) was retained in the colon for 30 s, after which the catheter was withdrawn.

Treatment with BDZ

The dose of the drug was adjusted using the equation developed by Reagan-Shaw et al. (2008). Bumadizone calcium dihydrate formula (containing 15 mg) were administered, using per-oral intubation in rabbits of all groups except the control group, once daily till (not including) the day of scarification.

Sample collection

About 1 mL blood sample was withdrawn from the ear vein of the rabbits. The samples were collected into heparinized test tubes at time intervals of 0 (predose), 1, 2, 3, 4, 6, 8 and 12 h following oral administration of a treatment. Plasma was separated by centrifugation at 3000 rpm for 10 min, collected in polyethylene-capped tubes and stored at $-20\,°C$ until analysis.

Quantitation of BDZ in plasma

A simple HPLC method (Schroeder & Stevens, 2003) was developed for the determination of BDZ in plasma. A Shimadzu HPLC system with UV–Visible detector: SPD-10 A, liquid chromatogram: LC-10AD, integrator: C-R6A, injector (20 µL): 6E (Shimadzu, Japan). The analysis was carried out using Hypersil C18 column (Thermo Electron Corporation) with dimensions $= 150 \times 4.6$ mm and particle size $= 5$ µm. The mobile phase consisted of acetonitrile:water (65:35) v/v, adjusted to pH 3.5 with phosphoric acid. The mobile phase solution was filtered and degassed. The flow rate was 0.8 mL/min. The eluent was monitored at 234 nm. Flufenamic acid was adopted as an internal standard. A standard calibration curve was constructed by plotting peak area ratio of BDZ and flufenamic acid against BDZ concentration in plasma. Validation parameters, namely, linearity, accuracy, recovery, interday and intraday precision were tested.

Pharmacokinetics analysis

The pharmacokinetic parameters of the two treatments were estimated for each subject these include: maximum drug concentration C_{max} (ng/ml), the time to reach C_{max}, T_{max}, $t_{1/2}$ and MRT.

Scarification of animals

Rabbits were sacrificed 5 days after the induction of colitis and a piece (5 cm) of colon was removed 8 cm from the rectum (El-kamel et al., 2008). The separated part was cleaned of fat and mesenteric blot, washed with 0.9% (w/v) saline solution and plotted on a filter paper.

Histopathological evaluation

Two tissue samples were excised from each colon and maintained in 10% (v/v) formalin in saline for histopathological evaluation. Sections of 5 µm were stained with hematoxylin and eosin (Kucharzik et al., 2001) and then examined using light microscope (Nikon, Tokyo, Japan) fitted with a Canon power shot G3 digital camera (Canon, Tokyo, Japan) and compared with control sample.

Measurement of myeloperoxidase activity

The activity of myeloperoxidase (MPO), which is found in neutrophils, can be used for evaluating the degree of

inflammation in the intestine. The distal colon specimen (200 mg) was minced in a beaker (cooled on ice) containing 10 times its amount water for injection, transferred to a test tube and homogenized at 1000 rpm (Potter E1 Vejhem glass homogenizer, Poland) three times for 30 s each. The homogenate (0.5 mL) was mixed with 0.5 mL of HTAB buffer (0.5% HTAB in 50 mM phosphate buffer, pH 6.0) and the mixture was sonicated for 10 s and centrifuged at 10 000 rpm for15 min. The supernatant was assayed for MPO activity. MPO activity was measured spectrophotometrically (UV-1601 PC spectrophotometer, Shimadzu, Kyoto, Japan): 0.1 mL of supernatant was combined with 2.9 mL containing 0.167 mg/mL O-diansidine hydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured every minute for 4 min. One unit of MPO activity is defined as the amount which degrades 1 mmol of the peroxide per minute at 25 °C (Cui et al., 1994; Kucharzik et al., 2001). The results were expressed as the mean ± standard deviation of the mean.

Results and discussion

Because of its simplicity and reproducibility, quasi-emulsion solvent diffusion method was used for the preparation of microspheres. Triethyl citrate was added as plasticizer and the external phase was saturated with 10% NaCl and 3% CaCl$_2$ in order to favor the drug entrapment inside the microspheres. Nine BDZ microspheres formulations were successfully prepared and subjected to evaluation and the results were as follows.

Scanning electron microscopy

Scanning electron microscopy of the dried microspheres revealed that the produced microspheres were spherical, non-aggregated with rough and porous surface (Figure 1). The pores might be induced by the diffusion of the solvent from the surface of the microspheres.
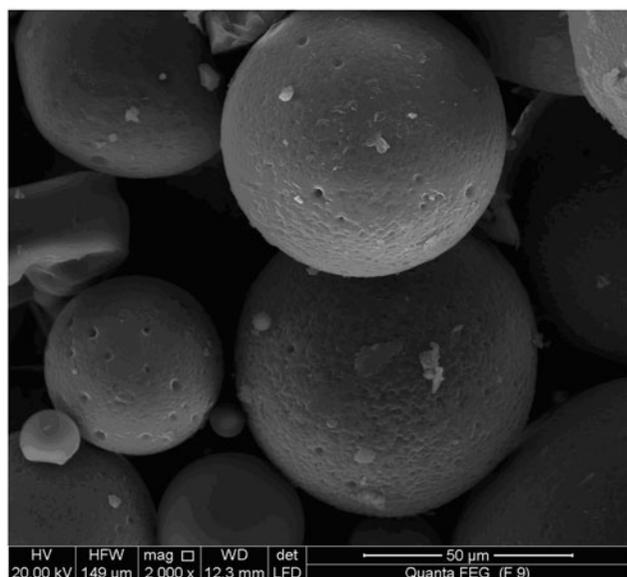

Figure 1. SEM of F9.

Particle size analysis

The mean particle size of the obtained microspheres ranged from 90.8 to 147.95 μm (Table 2). Both studied factors, namely, polymer type and drug:polymer ratio had non-significant effect on the mean particle size $p > 0.05$ (data not shown). This might be explained on the fact that particle size distribution of the microspheres was more dependent on the volume of dichloromethane and PVA concentration rather than on drug:polymer ratio (Lakshmana et al., 2009).

Production yield and entrapment efficiency

Results in Table 2 revealed that the production yield of the prepared microspheres varied from $45.45 \pm 5.23\%$ (F7) to $80.2 \pm 1.04\%$ (F9) while their entrapment efficiency was in the range of $44 \pm 10.36\%$ (F1) to $99.77\% \pm 1.25\%$ (F3).

The effect of polymer type

Polymer type had a significant effect ($p < 0.05$) on the percentage yield (Figure 2). It was found that CAB had lower percentage yield when compared to the other two polymers used. Jelvehgari & Montazam (2012) reported that CAB led to lower yield of theophylline microspheres when compared to Eudragit RS100. Similarly, polymer type had a significant effect ($p < 0.05$) on the entrapment efficiency. Microspheres prepared using CAB showed lower entrapment efficiency when compared to the other two polymers. This could be due to the high-permeability characteristics of CAB which would facilitate the diffusion of part of the entrapped drug to the surrounding medium during the preparation of the microspheres (Jelvehgari & Maghsoodi, 2010). On the other hand, microspheres prepared with ethyl cellulose showed the highest entrapment efficiency when compared to the other two polymers. This may be due to higher viscosity obtained in case of ethyl cellulose N100 when compared to Eudragit RS100 and CAB, decreasing drug diffusion to the outer phase and forcing it to stay in the microspheres formed resulting in higher entrapment. These findings are in accordance with the results reached by Swetha et al. (2011) who formulated etodolac microspheres using ethyl cellulose and Eudragit RS100 as polymers.

The effect of drug to polymer ratio

Increasing polymer to drug ratio led to a significant increase in production yield ($p < 0.05$) (Figure 2) This could be attributed to a slight increase in the inner phase viscosity providing more time for the droplet formation and improving yield also might lead to reduced diffusion rate of dichloromethane from concentrated solutions into aqueous phase. Our results are in accordance with Baykara (2003) who found that increasing drug to polymer ratio with keeping amount of the polymer constant led to an increase in production yield. Similarly, the entrapment efficiency of the prepared microspheres was found to increase significantly with increasing drug:polymer ratio. The higher drug entrapment efficiencies were obtained at the higher drug: polymer ratios (F3, F6 and F9). This may be because increasing drug loading would increase the drug concentration difference between that in the microsphere and in the surrounding

Figure 2. Main effects of the different factors according to 3^2 factorial design on the yield and entrapment efficiency: polymer type (A) and drug:polymer ratio (B).
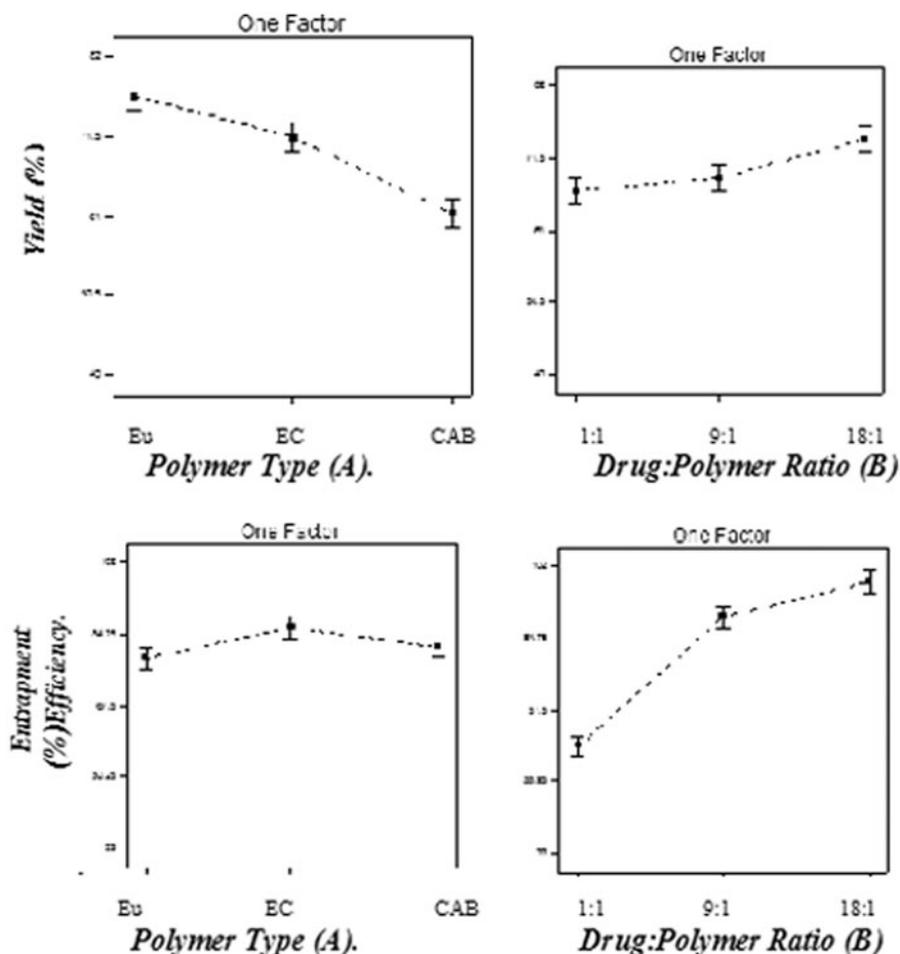
release medium which increase the driving drug release force, another reason could also be that the use of the higher amounts of BDZ when preparing microspheres caused slightly an increased viscosity of the dispersed phase. Increasing the viscosity of the internal media decreased the drug diffusion from the formed microspheres giving the drug enough time to be entrapped inside the particles formed. When dichloromethane diffused out, nearly all of the dispersed phase was converted to the form of solid microspheres and separated particles appear, furthermore, the highest drug entrapment efficiency of these formulations could be explained through the fact that the amount of drug in per unit polymer is greater than that in other formulations (Jelvehgari et al., 2006).

Differential scanning calorimetry

The DSC thermogram of pure BDZ showed that an exothermic peak associated with crystal melting at 158 °C (Figure 3). On the other hand, the amorphous blank polymer did not show any fusion peak or phase transition, but showed a broad signal around 144–166 °C. The DSC thermogram of BDZ-loaded CAB microspheres (F9) showed a broad signal around 63.94–88.19 °C. The peak corresponding to the melting point of BDZ disappeared. This could be attributed to the possible change of the drug to an amorphous form. To confirm the previous suggestions, X-ray diffraction studies were conducted.
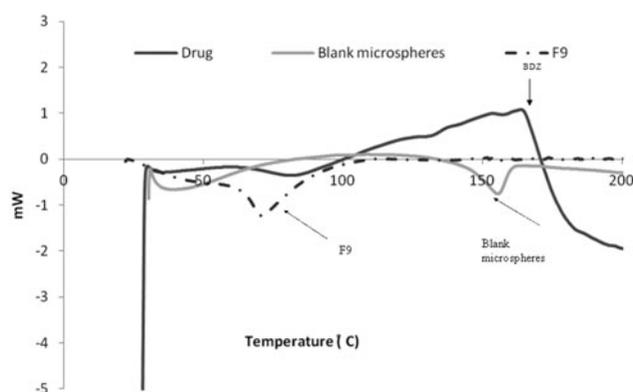

Figure 3. DSC thermograms of pure drug, blank microspheres and BDZ-loaded microspheres (F9).

X-ray diffraction

X-ray diffraction studies were done to investigate the crystalline status of the samples. The peak position (angle of diffraction) is an indicative of a crystal structure and peak heights are the measures of samples crystallinity in a diffractogram (Figure 4).

The diffractogram of BDZ shows three intense peaks at three different θ of 5.8°, 15.41° and 20.31°. The respective signal heights were 4080.31, 147.43 and 516.7, respectively. This was indicative of the crystallinity of the pure drug. While the diffractogram of plain microspheres shows three different
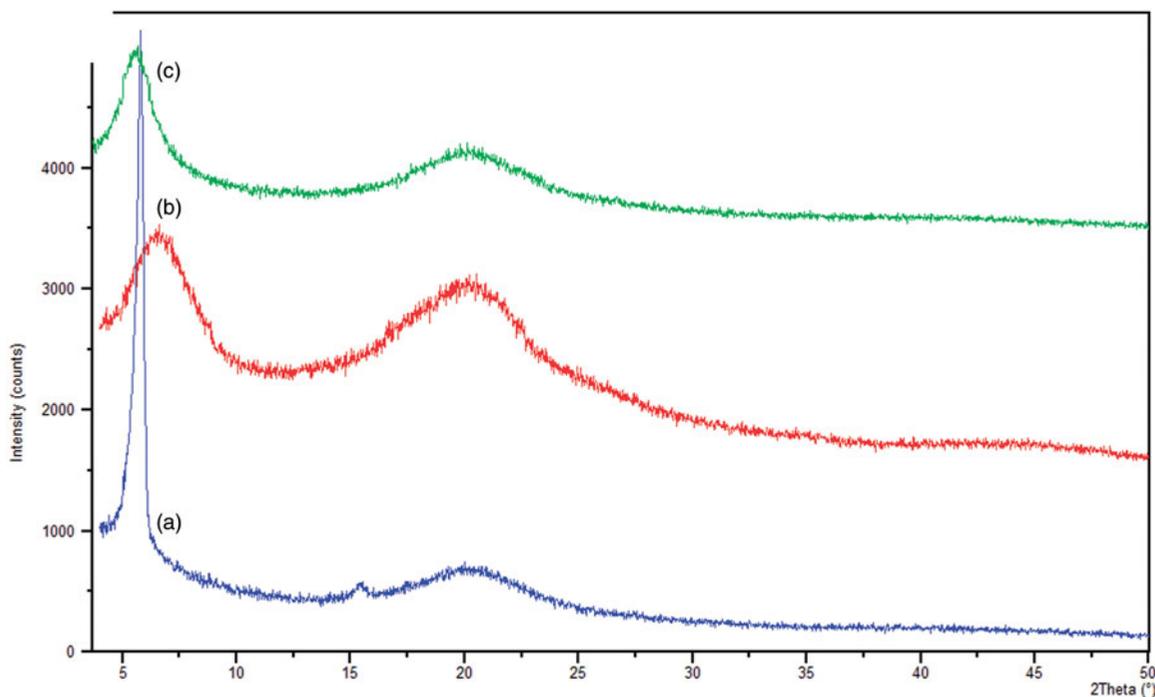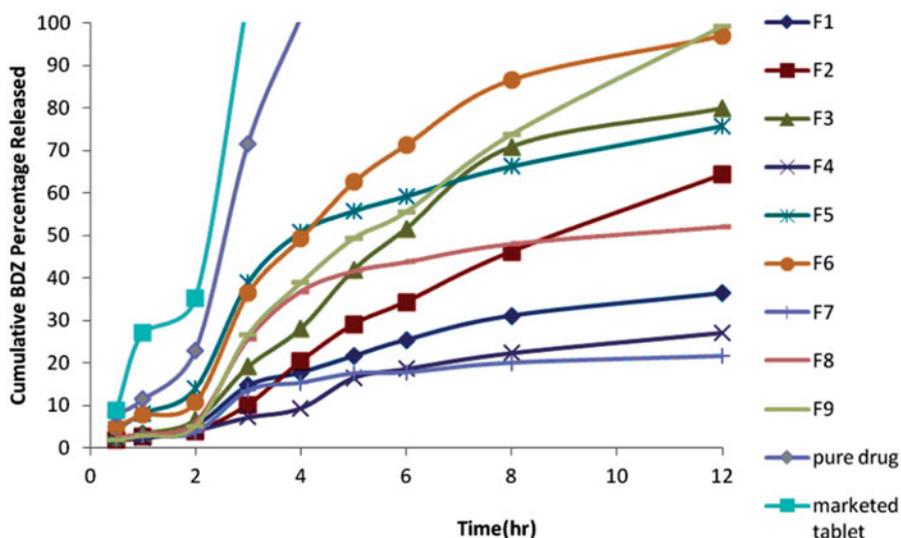
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Figure 4. X-ray diffractograms of (a) pure drug, (b) blank microspheres and (c) BDZ-loaded microspheres (F9).

Figure 5. Release profile of BDZ from the prepared microspheres in gradient pH system (pH 1.2 for 2 h, pH 6.8 for 2 h and pH 7.4 till the end of 12 h).

peaks and three different angles namely 6.56°, 20.175° and 44.45°. The respective signal heights were 1564.98, 1435.08 and 102.88, respectively. The loaded microspheres (F9) had two different peaks and two different angles, namely, 5.584° and 20.29° with heights of 1121.37 and 545.49, respectively. These results showed that a decrease in intensity of the peaks of the pure drug which favored the entrapment of the drug in the formed microspheres and changing of the drug from crystalline to amorphous form.

In vitro release studies of BDZ from the microspheres formulations

The *in vitro* release of the drug was done on a gradient pH basis so as to mimic the *in vivo* conditions (Saboji et al., 2012). Release profile of BDZ from the prepared microspheres are shown in (Figure 5). The results revealed that the

marketed bumadizone tablet and bumadizone powder showed fast dissolution when compared to the prepared microspheres as they showed 100% release after 3 and 4 h, respectively. The percentage of BDZ released from the prepared microspheres was found to be low for the initial 2 h in 0.1 N HCl, it was found to vary between 3.71% for F7 prepared using CAB in a drug to polymer ratio of (1:1) and 14.11% for F5 prepared using ethyl cellulose in a drug to polymer ratio of (9:1). Upon replacing 0.1 N HCl with phosphate buffer pH 6.8 for another 2 h, the percentage of BDZ released increased to 15.35% and to 50.82% for F7 and F5, respectively. On the other hand, the total BDZ released after 12 h from the prepared microspheres varied between 21.63% for F7 prepared with CAB in a drug:polymer ratio (1:1) and 99.1% for F9 prepared with CAB in a drug:polymer ratio of (18:1). To evaluate the effect of polymer type and drug:polymer ratio on the release profile, statistical analysis was done using design

expert software. (Design- Expert trial version 7; State-Ease Inc., Minneapolis, MN, USA). Both factors had a significant effect on the total percentage released after 12 h ($p < 0.05$).

Effect of drug:polymer ratio

Increasing drug:polymer ratio led to an increase in percentage of the drug released. This may due to that there was more drug than the polymer in the formed microspheres which led to an increase in entrapment efficiency and more homogenous drug distribution and consequently higher release. Also decreasing the polymer concentration in the formed microspheres, might facilitate drug diffusion to the release media. The increase in the rate and extent of drug release upon decreasing the polymer concentration might be due to the hydrophobic character of BDZ which hindered its delivery through the hydrophobic coat. Therefore, the diffusion of BDZ through the polymer wall became the controlling step of drug release. Delay in drug release at higher polymer concentration could be also attributed to the formation of thicker coating membranes, where 1:1 drug to polymer ratio contains 50% polymer, increasing drug concentration decreased polymer amount in formed microspheres as 9:1 ratio contains 90% drug and 10% polymer and 18:1 ratio contains 94.7% drug and 5.3% polymer.

Effect of polymer type

The used time-dependent polymers, namely, Eudragit® RS 100, Ethyl cellulose and CAB are known to be of hydrophobic nature, insoluble in water and buffers and are mainly used for sustainment of drug release (Rowe et al., 2006). In this study, ethyl cellulose and CAB showed faster release when compared to Eudragit® RS100. This could be attributed to the difference in permeability between the three polymers. Eudragit® RS 100 are copolymers of methacrylic acid and methyl methacrylate with known low permeability. Kotagale et al. (2011) reported that ranitidine hydrochloride microspheres prepared using ethyl cellulose showed higher and faster release if compared to microspheres prepared using Eudragit® RS 100 or combination of both ethyl cellulose and Eudragit®. Another reason for the slow release of BDZ from Eudragit® RS100 when compared to ethyl cellulose and CAB could be a possible interaction between the positively charged Eudragit RS100 and the negative charge of the drug.

Although F3, F6 and F9 showed higher % of drug released after 12 h which was 79.81, 96.98 and 99.1%, respectively. Unfortunately, none of the developed microspheres showed the desired drug release profile which must be less than 20% before target area (pH 1.2 and 6.8) so further optimization was required.

The aim of optimization of pharmaceutical dosage formulation is generally to determine the levels of variables from which a robust product with high quality characteristics may be produced. Design Expert® software was used to determine the best desirability for further optimization. The criteria selected were maximizing the yield, entrapment efficiency and total drug released after 12 h and minimizing the percentage of BDZ released before target area (pH 1.2 and 6.8). It excluded particle size as it was statistically insignificant.

Formulae F6 and F9 were chosen (Table 2) with a desirability of 0.963 and 0.872, respectively, for further optimization (to be compressed into tablets in order to decrease the percentage of drug released in pH 1.2 and 6.8). It is well known that desirability increases as this number closes to 1.

Pre-compression parameters

The value of angle of repose of the powders (different core and coat blends) ranged from $19.91 \pm 2.02°$ to $23.32 \pm 1.02°$ indicating good flow properties. The angle of repose is considered as a characteristic of the internal friction or cohesion of the particles. Powders with angle of repose above 50° have unsatisfactory difficult flow properties, 25–40° reasonable flow potential, whereas minimum angles close to 25° correspond to very good flow properties (Jones, 1988).

Post-compression parameters

All prepared tablet formulations met the British Pharmacopeia (Ivy Leaf, 2007) requirements for drug content and weight variation. The average drug content of the prepared formulations was found to be $101.44\% \pm 1.25$ while the average thickness ranged from 4.47 ± 0.85 to 4.79 ± 0.3 mm. None of the formulated tablets deviated from the average weight (500 mg) by more than 5%, indicating that all formulae fulfilled the pharmacopoeial specifications for weight variation.

According to the British Pharmacopeia hardness values between 4–6 and friability value <1% are considered acceptable. Hardness of the prepared tablets was within the acceptable range except F10 and F13 that had hardness values of 2.89 and 3.36 kg. The friability of the prepared tablets was within the compendial limits except F10 and F13 which had friability of 1.47 and 1.37%, respectively, that exceeded the pharmacopoeial limitation.

In vitro release of BDZ from compression coated tablets

The release profiles of BDZ from the compression coated tablets in pH gradient system are shown are shown in Figure 6.

Compression of the microspheres into coated tablets led to a decrease in the percentage of drug released in pH 1.2 and 6.8 when compared to the drug released from the corresponding microspheres (Figure 5).

All prepared tablets showed less than 20% of BDZ released in pH 1.2 and 6.8. F12, F14 and F15 showed 66.23, 67.9 and 79.42% of BDZ released after 12 h, respectively. This decrease in drug release might be attributed to the presence of pectin that acted as an additional barrier in front of the drug release before target area. Pectin may also have enabled drug targeting due to its bioactivity (Allena et al., 2012).

To evaluate the effect of core polymer type and pectin concentration in the coat on the total percentage of drug released, statistical analysis was done using Design Expert® software. Both factors had a significant effect ($p < 0.05$) on the total percentage released after 12 h.

Drug released from the compression-coated tablets increased as the percentage of pectin in the coat decreased. This might be because polymer (pectin) becames hydrated

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Figure 6. Release profile of BDZ from compression-coated colon-targeted tablets in gradient pH system (pH 1.2 for 2 h, pH 6.8 for 2 h and pH 7.4 till the end of 12 h).
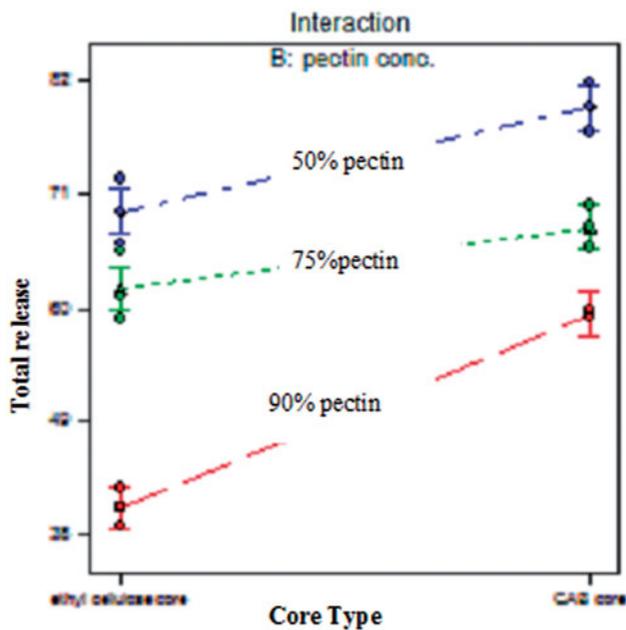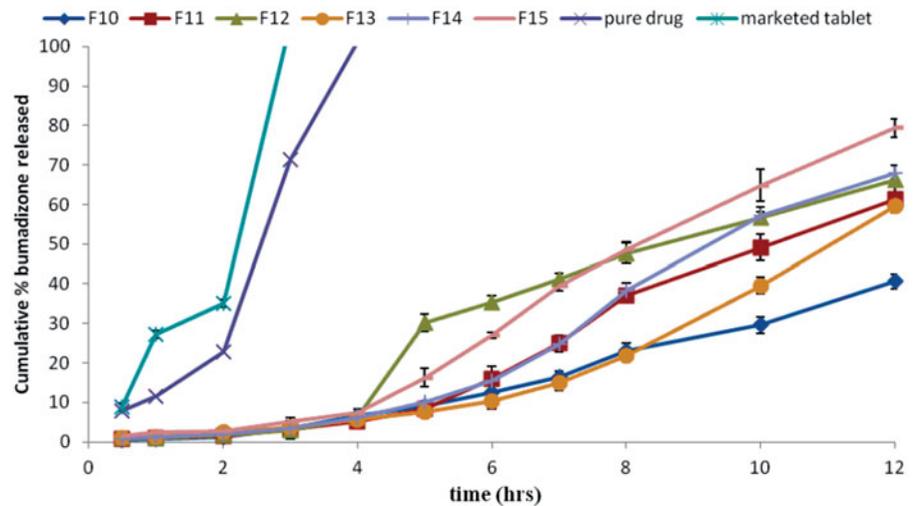

Figure 7. Interaction line plots showing the effect of core type and pectin concentration on the total percentage of drug released after 12 h.

and forms a viscous gel layer that slowed down further seeping-in of dissolution fluids towards the core tablets (Patel et al., 2010). The interaction line plots (Figure 7) show that the effect of the polymer type varied according to the pectin concentration used. At different pectin concentration, CAB showed higher drug release when compared to ethyl cellulose. This effect was prominent at 90% pectin but less clear at lower pectin concentrations. This could be attributed to higher permeability characteristics and lower viscosity of CAB when compared to ethyl cellulose. Ethyl cellulose is one of the most widely used water-insoluble polymers in film-coating to achieve extended drug release. In order to evaluate enzyme-triggered drug release of pectin compression-coated tablets, 7.4 to simulate the degradation of the polymers by the microflora (Turkoglu & Ugurlu, 2002).

Using Design Expert software, F12 and F15 were selected and their release was repeated with the addition of 1 ml of pectinolytic enzyme to Sorensen's buffer (pH 7.4; F12P and F15P). Results are illustrated in (Figure 8). The results revealed that there was an increase in release of the drug in target area pH 7.4 in the presence of pectinolytic enzyme that hydrolyzed pectin in the coat formulation. The presence of pectinolytic enzyme in pH 7.4 led to hydrolysis of the pectin of the coat, hence decreasing the barrier in front of the drug together with forming channels in the coat. This in return allowed the release media to reach the core, releasing the drug (Okafor et al., 2010). F15 achieved the lowest percentage of drug released before target area (pH 1.2 and 6.8) of 7.26% and maximum percentage of drug released in target area (pH = 7.4) of 72.16%. After the addition of 1 mL pectinase enzyme the percentage of BDZ released increased from 72.16% to 99.7% after 12 h.

Kinetic analysis of the release profiles of the compression-coated colon-targeted tablets are shown in Table 4. In all tested formulations, calculated n values were >0.89 indicating a super case II release. A super case II release mechanism usually indicates that polymer erosion is the technique involved in the release of the drug. It occurs when the erosion rate of the polymer is higher than its swelling rate. Regarding F12 P and F15P, the presence of pectinase enzyme aided to dissolve the pectin present in the coat blend (Arunachalam & Asha, 2010) and this is the main reason for the use of pectin in colon targeting (El-Gamal et al., 2013). It was previously reported that a super case II release is the expected mechanism when the polymer dissolves in the release media (Costa et al., 2001).

In vivo evaluation

Plasma-time curve

Plasma concentration–time curve was investigated to confirm the ability of the candidate formula to prevent or minimize drug absorption before target area and to sense the arrival of the dosage form to the colon where drug release should start and be maintained up to the 12th hour.

An HPLC method was adopted (Schroeder & Stevens 2003), a calibration curve was constructed and a linear

Figure 8. *In vitro* release of BDZ from prepared formulations (F12 and F15) in the presence (F12P and F15P) and in the absence (F12 and F15) of pectinase enzyme.
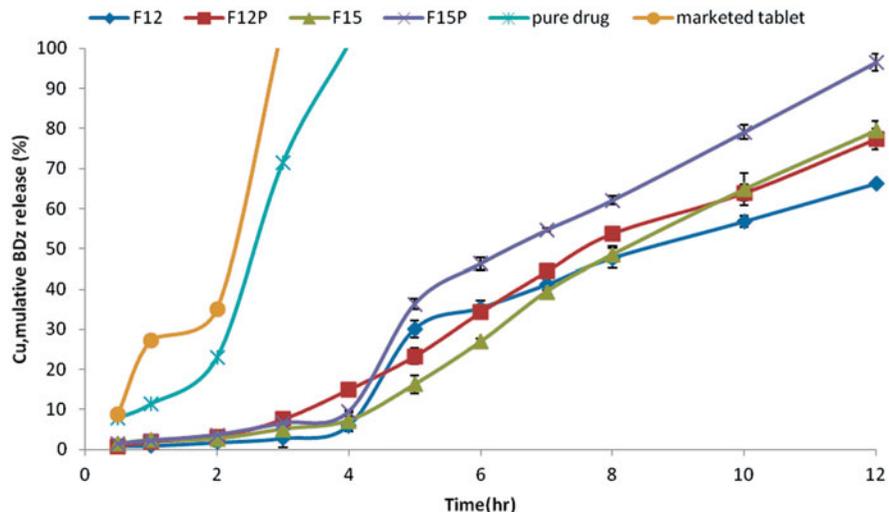

Table 4. Mathematical modeling and release kinetics of BDZ from the compression-coated colon-targeted tablets.

| Formula code | Correlation coefficient (R^2) | | | Korsmeyer–Peppas model | | | |
	Zero order	First order	Higuchi model	Correlation coefficient (R^2)	Release exponent (n)	Order of release	Case order
F10	0.9575	0.9066	0.8497	0.9564	1.4494	Zero	Super case II
F11	0.9236	0.9413	0.800	0.9034	1.5078	First	Super case II
F12 P*	0.9416	0.8462	0.8871	0.8686	1.6288	Zero	Super case II
F13	0.8551	0.9745	0.709	0.9291	1.33	First	Super case II
F14	0.9115	0.950	0.7827	0.9207	1.4822	First	Super case II
F15 P*	0.9486	0.9301	0.8465	0.8747	1.2933	Zero	Super case II

P*: release was done in presence of pectinase enzyme.

Table 5. Pharmacokinetic parameters of BDZ following oral administration of octomotol and the prepared colon targeted tablets (F15).

| | The tested formulae | |
Parameter	Octomotol® tablets	F15
Cp_{max} (ng/mL)	5110.3 ± 103.21	2700.2 ± 135.14
T_{max} (h)	1	4
MRT (h)	2.49	5.91
Kel (h^{-1})	$0.16 + 0.008$	$0.102 + 0.0047$
$T_{1/2}$ (h)	$4.286 + 0.235$	$6.79 + 0.31$
AUC (0–12) ng.h/ml	$11\,898 + 385.16$	$10\,443.15 + 671.63$

response ($r^2 = 0.998$) across the full range of concentrations from 100 to 1500 ng/ml was achieved, satisfactory validation parameters, namely, linearity, accuracy, recovery percentage, interday and intraday precision were obtained.

The pharmacokinetic parameters of bumadizone calcium dihydrate following oral administration of a single dose (15 mg) of (i) marketed product and (ii) F15 are shown in Table 5.

The drug plasma concentration–time profiles of both treatments are depicted in (Figure 9). It is clear that remarkable differences between the two treatments were found. The peak plasma concentration (C_{max}) of both treatments were found to be 5110.3 ± 103.21 (ng/ml) and 2700.2 ± 135.14 (ng/ml) for the marketed product Octomotol® tablets and the candidate formula (F15), respectively. Statistical analysis of C_{max} revealed significant difference ($p < 0.05$) where candidate formula showed lower C_{max} in comparison to the marketed product. T_{max} was 1 and 4 h for marketed product and candidate

formula, respectively (Table 5). These results confirmed that the candidate formula was able to delay the drug absorption (T_{max} 4 h) till the 4th hour (minimum drug absorption at the upper parts of GIT) and to sense the arrival of the dosage form to the target area (colon) when compared to the marketed product that showed a lower T_{max} of only 1 h. This could be further confirmed by the increase of the MRT from 2.49 h of the marketed product to 5.91 h of the candidate formula. All the above-mentioned pharmacokinetic parameters (C_{max}, T_{max} and MRT) were statistically significant ($p < 0.05$) and correlate well with the *in vitro* release data.

Histopathological analysis

Histopathological examination of the obtained animal tissue revealed that acetic acid was able to induce a severe inflammation in the colon tissue. This resulted in a pan mural inflammation together with ulceration and gangrene if compared to normal tissue. Treatment with the marketed product did not show significant decrease in formed ulceration and inflammation. However, treatment with F15 showed a marked decrease in inflammation and neutrophil infiltration (Figure 10).

MPO measurement

The acetic acid induced experimental rabbit colitis model was selected because the pharmacological responses of rabbit colonic smooth muscle to inflammatory mediators closely resemble those of the human colon (Percy et al., 1990). MPO activity, which is an important quantitative index for colonic

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Figure 9. Mean plasma concentrations of BDZ following administration of an oral dose of 15 mg of Octomotol® tablets and F15 compression-coated colon-targeted tablets to three rabbits.
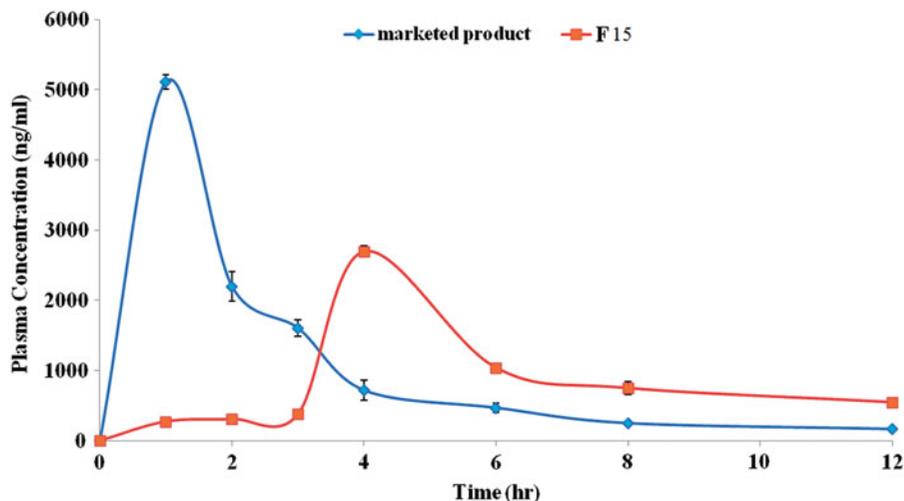

Figure 10. Histopathological analysis of colon tissue. (a) Normal mucosa. (b) Acetic acid induced colitis:pan hemorrhagic infarction, marked inflammation. (c) Treatment with marketed product showing neutrophil infiltration. (d) Treatment with F15: marked decrease in inflammation and neutrophil infiltration.
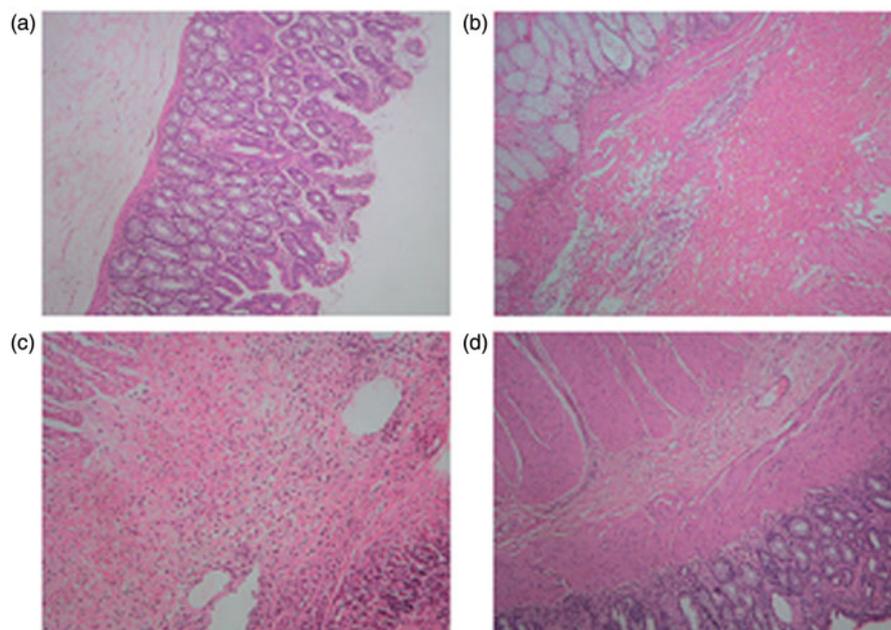

Table 6. Mean myeloperoxidase activity of treated and untreated animals (mean $\pm$ SD, $n = 3$).

Animal group	MPO activity (Mean $\pm$ SD, $n = 3$)
Normal	2.724 ± 0.809
Acetic acid induced colitis	15.363 ± 1.205
Treatment with marketed product	11.96 ± 1.30
Treatment with F15	5.7 ± 0.389

inflammation, was determined in terms of units per gram tissue weight (Soad et al., 2009). MPO activity for normal control group was 2.724 ± 0.809 U/g tissue weight. However, MPO activity of the induced colitis group was found to be 15.363 ± 1.205 U/g tissue weight. This MPO was significantly decreased to 11.96 ± 1.30 and 5.7 ± 0.389 U/g tissue weight in the case of the group that received the marketed product and F15, respectively (Table 6). Statistical analysis was done using SPSS statistical package version 17 (SPSS Inc., Chicago, IL). *Post-hoc* test revealed that the groups are statistically significant ($p < 0.05$) concerning MPO activity.

Conclusion

BDZ was successfully colon targeted using the techniques adopted. Candidate formula (F15) (microspheres prepared using BDZ:CAB in a ratio of 18:1 and compressed into tablets using 50% pectin and 50% Avicel in the coat) was able to adequately modulate drug release avoiding drug release in the gastric ambient, and reaching the colonic targeting where 99.7% release was achieved within 12 h following the zero-order model. Histopathological investigation showed a marked decrease in inflammation and colorimetric assay of inflammatory marker (Myelo peroxidase enzyme) revealed its efficacy when compared to the marketed product. Statistical analysis revealed significant increase in T_{max} (4 h) and in MRT (5.91 h) with a decrease in C_{max} (2700 ng/ml) when compared to marketed product. This indicates decreased and delayed absorption of the drug. These findings suggest that the microspheres compressed into tablets containing BDZ (F15) are likely to become one of choices for the treatment of ulcerative colitis.

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Declaration of interest

No conflict of interest.

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